

The following Listing of the Claims will replace all prior versions and all prior listings of the claims in the present application:

Listing of The Claims:

1. (Currently Amended) A method of molecular cloning, wherein a nucleic acid insert molecule is covalently joined to first and second double stranded nucleic acid flanking molecules to form a ligated molecule, the method comprising:

(A) incubating said insert molecule and said flanking molecules, wherein each end of said insert molecule comprises a 5'-hydroxyl group, and wherein one end only of each of said first and second flanking molecules comprises a covalently bound topoisomerase polypeptide, under conditions which permit their covalent joining to form a ligated molecule comprising a said insert molecule positioned between a said first and a said second flanking molecule; and

(B) transforming the covalently joined insert molecule of step (a) into a host cell to obtain transformants.

2. (Withdrawn) A method of covalently joining a nucleic acid insert molecule to first and second nucleic acid flanking molecules to form a ligated molecule, the method comprising

(A) incubating:

said insert molecule, wherein one end of said insert molecule comprises a 5'-hydroxyl group and the other end comprises a 5'-phosphate group, and

a said first flanking molecule, wherein one end only of said first flanking molecule comprises a covalently bound topoisomerase polypeptide, under conditions which permit their covalent joining to form a ligated nucleic acid comprising a said insert molecule positioned adjacent to a said first flanking molecule,

(B) incubating a said ligated nucleic acid of step (A) with phosphatase under conditions which permit removal of a 5'-phosphate group from said ligated nucleic acid; and

(C) incubating a product of step (B) with a said second flanking molecule, wherein one end only of said second flanking molecule comprises a covalently bound topoisomerase polypeptide, under conditions which permit covalent joining to form a ligated molecule comprising a said insert molecule positioned between a said first and a said second flanking molecule.

3. (Withdrawn) A method of covalently joining a nucleic acid insert molecule to first and second nucleic acid flanking molecules to form a ligated molecule, the method comprising

incubating:

said insert molecule and said flanking molecules, wherein one end of said insert molecule comprises a 5'-hydroxyl group and the other end comprises a 5'-phosphate group, wherein one end only of said first flanking molecule comprises a covalently bound topoisomerase polypeptide and wherein one end of said second flanking molecule comprises a ligase substrate site, under conditions which permit their covalent joining to form a ligated molecule comprising a said insert molecule positioned between a said first and a said second flanking molecule.

4. (Previously Presented) The method of claim 1, wherein a said first and a said second nucleic acid flanking molecules comprise a left and a right vector arm, respectively, such that a said insert molecule is flanked by a said left vector arm and a said right vector arm.

5. (Previously Presented) The method of claim 4, wherein said left and right vector arms each comprise a free end that is not joined to an insert molecule, said method further comprising the step of:

joining the free ends of said vector arms to each other by a method selected from the group consisting of nucleic acid ligase mediated ligation, complementary sequence annealing, topoisomerase mediated ligation, *in vitro* site-specific recombination, *in vivo* site-specific recombination, and *in vivo* homologous recombination.

6. (Currently Amended) A method of molecular cloning comprising:

(A) incubating a nucleic acid insert molecule comprising a 5'-hydroxyl group at one end and a 5'-phosphate at the other end, and a linear double stranded cloning vector, wherein the linear cloning vector comprises a covalently bound topoisomerase polypeptide at one end only and a ligation substrate site at the other end, under conditions sufficient for the covalent joining of said insert to said vector to form a ligated circular vector comprising said linear cloning vector and said insert molecule; and

(B) transforming the ligated circular vector of step (A) into a host cell to obtain transformants.

7. (Withdrawn) A method for molecular cloning comprising:

(A) incubating a nucleic acid insert molecule, wherein each end of said insert molecule comprises a 5'-hydroxyl group, and a first and a second linear arm wherein one end only of each of said first and second linear arms comprises a covalently bound topoisomerase and the other end comprises a cloning substrate site, under conditions sufficient for their covalent joining to form a ligated molecule comprising a said insert molecule positioned between a said first and a said second linear arm; and

(B) transforming the ligated insert molecule of step (A) into a host cell to obtain transformants.

8. (Withdrawn) The method of claim 7 wherein said cloning substrate site is selected from the group consisting of a *cos* site, a LIC site, and a loxP site.

9. (Withdrawn) The method of claim 7 wherein said cloning substrate site is loxP and wherein said incubating step further comprises incubating *in vitro* said ligated molecule with a Cre recombinase and a circular plasmid comprising a loxP site, under conditions sufficient for site-specific recombination to form a circular plasmid comprising said ligated molecule, wherein said circular plasmid comprising said ligated molecule is transformed into a host cell.
10. (Withdrawn) The method of claim 7 wherein said cloning substrate site is loxP and wherein said transforming step further comprises transforming said ligated molecule into a host cell comprising a circular plasmid comprising a loxP site, wherein said cell expresses Cre recombinase, under conditions sufficient for site-specific recombination to form a circular plasmid comprising said ligated molecule within said cell.
11. (Withdrawn) The method of claim 7 wherein said cloning substrate site is a site for homologous recombination with a circular plasmid vector, wherein said transforming step further comprises transforming said ligated molecule into a host cell comprising a circular plasmid vector, wherein said circular plasmid vector comprises a site for homologous recombination with said ligated molecule, wherein said host cell is recA+, under conditions sufficient for homologous recombination to form a circular plasmid comprising said ligated molecule within said host cell.
12. (Withdrawn) The method of claim 7 wherein said first linear arm comprises a left lambda arm comprising at one end only a covalently bound topoisomerase, and wherein the second linear arm comprises a right lambda arm comprising at one end only a covalently bound topoisomerase.
13. (Withdrawn) A method for molecular cloning comprising:
- (A) incubating a nucleic acid insert molecule, wherein one end of said insert molecule comprises a 5'-hydroxyl group and the other end comprises a 5'-phosphate group, and a first linear arm, wherein one end only of said first linear arm comprises a covalently bound

topoisomerase polypeptide and the other end comprises a cloning substrate site, under conditions which permit their covalent joining to form a ligated insert/first linear arm molecule;

(B) incubating a said ligated insert/first linear arm molecule of step (A) with phosphatase under conditions which permit removal of a 5'-phosphate group to form a product which comprises a said ligated insert/first linear arm molecule that lacks a 5' phosphate; and

(C) incubating said product of step (B) with a second linear vector arm, wherein one end only of said second linear vector arm comprises a covalently bound topoisomerase polypeptide and the other end comprises a cloning substrate site, under conditions which permit covalent joining to form a ligated molecule comprising a said insert molecule positioned between a said first and a said second linear vector arm;

(D) transforming the ligated molecule of step (C) into a host cell to obtain transformants.

14. (Withdrawn) The method of claim 13 wherein said cloning substrate site is selected from the group consisting of a *cos* site, a LIC site, and a loxP site.

15. (Withdrawn) The method of claim 13 wherein said cloning substrate site is loxP and wherein said ligated molecule is further incubated *in vitro* with a Cre recombinase and a circular plasmid comprising a loxP site, under conditions sufficient for site-specific recombination to form a circular plasmid comprising said ligated molecule, wherein said circular plasmid comprising said ligated molecule is transformed into a host cell.

16. (Withdrawn) The method of claim 13 wherein said cloning substrate site is loxP and wherein said transforming step further comprises transforming said ligated molecule into a host cell comprising a circular plasmid comprising a loxP site, wherein said cell expresses Cre recombinase, under conditions sufficient for site-specific recombination to form a circular plasmid comprising said ligated molecule within said cell.

17. (Withdrawn) The method of claim 13 wherein said cloning substrate site is a site for homologous recombination with a circular plasmid vector, wherein said transforming step further comprises transforming said ligated molecule into a host cell comprising a circular plasmid vector, wherein said circular plasmid vector comprises a site for homologous recombination, wherein said host cell is recA+, under conditions sufficient for homologous recombination to form a circular plasmid comprising said ligated molecule within said host cell.

18. (Withdrawn) The method of claim 13 wherein said first linear arm comprises a left lambda arm comprising a covalently bound topoisomerase polypeptide at one end, and wherein the second linear arm comprises a right lambda arm comprising a covalently bound topoisomerase polypeptide at one end.

19. (Withdrawn) A method for molecular cloning comprising:

(A) incubating:

a nucleic acid insert molecule, wherein one end of said insert molecule comprises a 5'-hydroxyl group and the other end comprises a 5'-phosphate group;

a first linear arm, wherein one end only of said first linear arm comprises a covalently bound topoisomerase polypeptide and the other end comprises a cloning substrate site; and

a second linear arm, wherein one end of second linear arm comprises a ligase substrate site and the other end comprises a cloning substrate site, under conditions which permit their covalent joining to form a ligated molecule comprising a said insert molecule positioned between a said first and a said second linear arm;

(B) transforming the ligated molecule of step (A) into a host cell to obtain transformants.

20. (Withdrawn) The method of claim 19 wherein said cloning substrate site is selected from the group consisting of a *cos* site, a LIC site, and a loxP site.

21. (Withdrawn) The method of claim 19 wherein said cloning substrate site is loxP and wherein said incubating step further comprises incubating *in vitro* said ligated molecule with a Cre recombinase and a circular plasmid comprising a loxP site, under conditions sufficient for site-specific recombination to form a circular plasmid comprising said ligated molecule, wherein said circular plasmid comprising said ligated molecule is transformed into a host cell.

22. (New) The method of claim 5 wherein said joining of the free ends of said left and right vector arms comprises *in vivo* site-specific recombination.

23. (New) The method of claim 22 wherein the free end of each of said left and right vector arms that is not joined to said insert molecule comprises a loxP site, and wherein said transforming step (B) comprises transforming the covalently joined molecule of step (A) into a host cell comprising Cre recombinase, wherein said transforming results in *in vivo* site-specific recombination between the free end of said left vector arm and the free end of said right vector arm to generate a circularized vector molecule.